Thesis/
Reports
Wiegrefe

Development of a Drought Screen for Woody Perennial Seedlings Using Polyethylene Glycol as an Osmoticum

Susan J. Wiegrefe and Harold Pellett

DEVELOPMENT OF A DROUGHT SCREEN FOR WOODY PERENNIAL SEEDLINGS USING POLYETHYLENE GLYCOL AS AN OSMOTICUM

The Rocky Mountain Station of the USDA-FS Challenge Cost Share Program

Final Report

Submitted by:

Susan J. Wiegrefe, Ph. D.
University of Minnesota
Department of Horticultural Science
Saint Paul, MN 55108

Harold Pellett, Ph. D.
University of Minnesota
Department of Horticultural Science
Saint Paul, MN 55108

LIBRARY COPY
ROCKY MTN. FOREST & RANGE
EXPERIMENT STATION

TABLE OF CONTENTS:

Introduction 3

System Specifications and Operation 4

Solution tanks and circulation system 4

Cooling and Insulation 5

Plant treatment and membrane envelopes 6

Solution preparation and changing 6

Table 1. 7

Time Line of Experiments and Findings 8

Fall 1993: Preliminary work 8

January - March 1994: Trial I 8

Summer 1994: Interim 9

September - October 1994: Trial II 9

Winter 1994 - 1995: Trial III 10

Conclusions 13

Recommended Design Changes for Future Studies 14

Literature Cited 16

Figures 17

INTRODUCTION:

Osmotica are soluble compounds which can be used to alter the water potential of a solution by changing the solute concentration. These compounds are useful research tools for evaluating plant responses to drought stress. Using osmotica, a known osmotic potential can be applied to a plant's entire root system and uniformly to many plants (13). This is more precise, quantifiable, and reproducible than the level of stress experienced by plants stressed by withholding water. Whether the latter system is conducted in a pot, bed, or in the ground, different portions of the plants' root systems are exposed to different moisture levels due to surface evaporation, gravity flow of water, and differential removal of water through transpiration. Furthermore, using osmotica the duration and intensity of the drought episode can be closely controlled.

Osmotica have been used for many years to impart precise levels of drought stress to plants and plant tissues. Polyethylene glycol (PEG) is a neutral polymer which has been used extensively due to its high solubility and low biological impact on plants relative to other osmotica, which are mostly salts and sugars (10,11,12; but see also 5). Some researchers place their plant materials in direct contact with PEG solutions and claim there is negligible uptake, or that the effects, if PEG is absorbed, are inconsequential (5,6). Others have detected toxic effects associated with direct PEG usage (4,7,8,9,12). Theories that these effects are the result of impurities resulting from the manufacturing process (9) were found not to be substantiated (7). The possible inhibition of phosphorous translocation by PEG could not be determined to be due to the compound itself as opposed to the osmotic potential imparted by it (12). However, evidence exists indicating that although intact roots excluded large molecular weight formulations of PEG, damaged roots or roots subjected to low osmotic potentials take up even large PEG molecules causing vascular blockage, reducing water absorption, and causing desiccation of the plant (7).

Additionally, both circumstantial evidence (9) and mathematical models (11) indicate that oxygen availability may be limiting in solutions with high PEG concentrations and high molecular weight formulations due to the high viscosity and low diffusion rate of oxygen in these solutions.

The intent of the current study was to design a drought screening system which would utilize the positive aspects of PEG use as an osmoticum, but which would minimize possible negative effects. To circumvent the problems associated with the uptake of PEG, semipermeable membranes have been utilized to allow diffusion of nutrients and water while restricting the influx of PEG into the root area. This strategy was first developed by Zur (15,16) and has been modified in various ways, usually for use on herbaceous plant materials, e. g., *Phaseolus spp.* (1,14) and *Vigna radiata* and *Oryza sativa* (13). Our goal in this study was to determine if it could be modified successfully for use with woody perennials in such a way that it could be feasibly employed for testing the large numbers of seedlings necessary in order to identify genotypes with superior drought tolerance from natural or control-crossed populations. Alternatively, its effectiveness in identifying specific components of drought tolerance was also to be evaluated. The relative merit of the system was to be gauged by comparison with the findings from parallel studies using

shared plant materials but alternative methods of drought tolerance screening done by collaborators at the USDA Forest Service, Center for Semiarid Agroforestry.

SYSTEM SPECIFICATIONS AND OPERATION:

A hydroponic system was designed with the main objectives being to: 1) minimize the solution volume (and thus, the cost of the PEG) required, 2) preclude plant uptake of PEG, 3) maximize agitation and aeration of the hydroponic solution, and 4) minimize microbial growth within the system. The ultimate goal was to be able to simulate a three to four week drought event using decreasing osmotic potentials.

SOLUTION TANKS AND CIRCULATION SYSTEM:

The plants are positioned in tall narrow troughs which are constructed of opaque, gray 1/4" thick sheets of "PVC-Schedule 40" (Figure 1). The exterior dimensions of each trough are 145 cm x 22 cm x 3.3 cm and are designed to accommodate twelve plants. The solution is delivered to each trough through a 1/2" hole near the bottom of one of the end panels. Towards the exterior this hole is fitted with a male connector and to the interior a piece of 3/4" ID PVC tubing is attached to the connector. This tubing runs the length of the trough and has 1/16" holes bored into the bottom of it at 12 cm intervals, beginning 6 cm from the intake end and is blocked at the far end. This is to ensure that equally-oxygenated solution is provided to each plant regardless of its position in the trough. The size of the holes is such that the solution is expelled under pressure, causing some turbulence of the surrounding solution. Because of this force, the far end of the tubing is affixed to the bottom of the trough with flowable silicone sealant to keep it at the bottom of the trough.

The solution level is maintained at a constant level by a 5/8" diameter hole drilled into the same endpanel as the intake hole and positioned with its center 1" from the top edge. This hole is fitted with a one-sided male connector. All seams in the troughs are sealed with PVC cement and a layer of underwater putty (Devcon). The bottom seams are reinforced with screws drilled through each of the sides and into the bottom panel at 12 cm spacings. Along the upper edge of the troughs are spacers which act as additional support for the seams in countering the outward pressure from the solution inside, as well as supports for the plant assemblages to rest upon. They are positioned 1.5 cm from the top of the troughs, (just above the water line) and consist of bolts passing from one side of the trough through one wall, hollow spacer rods, and through the other wall, where they are fastened with a nut.

A 18" piece of 3/4" ID black opaque Norprene tubing (Norton Co.; Cole Parmer #M-06410-18) is attached to the outside of the overflow hole and allows the excess solution to drop through holes cut in the fitted top of a large HDPE tub (Cole Parmer M-06810-70 and -75). The tub's capacity is in excess of nine gallons, and is thus capable of holding solution for four troughs and all the extra solution required to keep the connective tubing filled as well. A fitting was installed (carefully) into the bottom front of the two solution

tanks. An amber latex tube (1/2" ID x 1/8" wall; later (for trial 3), this was replaced with a more rigid tubing of silastic (1/2" ID x 1/8" wall) wrapped with duct tape to exclude light) transfers the solution by gravity to the pump.

The operation of the each single-phase pump (Little Giant 581-1935) is regulated by a flow meter (Dwyer RMC-1435SV). After passing through the flow meter the solution arrives at a manifold (custom made) which divides the stream into four lines, which are attached via hose clamps to the intake connectors on the troughs. The tubing from the pump to the flow meter, and from the flow meter to the troughs is 3/8" ID opaque black latex.

Two sets of troughs are each held upright and parallel using 2" x 8" joist hangers attached to two 2" x 4" sections with the centers of the troughs 8" apart. Wires attached to the holes on one end of the joist support, passing across the tops of the troughs, and secured in the joist support arm on the opposite side stabilize each trough. The troughs are elevated above the height of the solution tubs by being placed , along with their 2" x 4" bases, on top of other HDPE tubs. The solution tubs, in turn are placed on crates to minimize impediments to the flow of solution to the pumps.

COOLING AND INSULATION:

Due to solution temperatures reaching as high as 38°C on hot, cloudless days, the following modifications were made in order to cool and insulate the solution. This was done to avoid the possible confounding effects of high root temperatures (4).

A water bath (Forma Scientific Model 2160; Figure 2) was used to refrigerate water passing through two heat exchangers, which were constructed to cool the solutions after passing through the flow meters and immediately before being dispensed into the troughs. Manifolds were built on to the end of the heat exchangers (Figure 3). All tubing is insulated and made as light exclusive as possible using foam tubing insulation. Thermocouples and thermostats (Model 70B, United Electric Controls Company, Watertown, MA) were installed to regulate when water is flowing through the heat exchangers. During the trial run a multichannel data recorder documented that the temperature of the solutions in the troughs could be maintained within 1°C of the set temperature of 20°C while ambient greenhouse temperatures fluctuated between 16 and 40°C.

In addition, 1" white "beadboard" (styrofoam insulation) sheets were cut to fit the broad sides of the troughs. The stabilizing joist supports were switched at the time of insulation installation to 3 x 9" to accommodate the additional width. The surfaces of the beadboard and the troughs were held together by heavy wires bent to exert inward force on the exterior of the beadboard. The beadboard came to the top of the solution level/ level of the spacers at the top of the troughs leaving the upper edges accessible for light-exclusion tape. (Figure 4).

Reservoir tubs were also shaded with white plastic.

PLANT TREATMENT AND ROOT ENVELOPES:

Plants were grown in either 50% Turface (ICM Chemical Group, Boston, MA): 50% sand, or 50% perlite: 50% vermiculite prior to the experimental treatment. These media were chosen because they are relatively easy to wash from the root system. Because neither of these media contain the necessary plant nutrients to sustain plant growth, the plants were irrigated alternately with water and half-strength Hoagland solution.

At the start of an experiment the media is washed from the plants' roots. Twenty centimeter lengths of SpectaPor #1, 120 mm tubing (Spectrum Medical Inc., Los Angeles, CA) were presoaked in deionized water and the bottom end of the tube is closed off. This was done by folding it over and pulling the open edge of a hollow plastic rod which has been cut/abraded to expose a slit of <2 mm in width over the fold (Figure 5). The effectiveness of this system for holding the fold closed and being "water-tight" was tested in the following manner. Two tubes were closed off in the described manner and suspended in a beaker of distilled water. PEG was added to the solution inside of one of the bags and the apparati were allowed to stand for 48 hours. At the end of that period the water outside the bags was tested using an osmometer (Wescor 5100C, Logan, Utah). The osmometer readings were identical for the control and the PEG bag.

The pre-labeled plant is placed inside the tubing with the previous soil line about 1 cm below the top edge. One half of a silicone stopper which has had a hole bored longitudinally down the center is placed inside the membrane with the half-bore around the stem of the plant to support and protect it. The whole assemblage is then placed against a 12 cm x 1.2 cm x 0.2 cm plexiglas strip and surrounded by a #84 rubber band stretched tight around the ends of the plexiglas strip. Two medium binder clips are then placed over the rubber band near the stopper. A special stand was constructed to enable one person to set up an experiment unassisted. (Figure 6).

The ends of the plexiglas strip extend beyond the membrane and balance on the spacer bars at the top of the trough, with the membrane immersed up to the rubber band in solution. The flared ends of the binder clips also serve to prevent the unit from falling into the trough. Once all the plants are positioned the spaces between the plants and the clips and between the clips of neighboring plants are covered with opaque tape to exclude light, which would inhibit root growth and promote microbial growth.

Two antimicrobial tactics were employed at different times in the testing of this system. The first entailed a dip of the plant roots in a solution of 750 ppm Phyton 27 (Source Technology Biologicals, Inc., Minneapolis, MN) and the second involved the pipetting of 0.08 ml of Subdue 2E (Ciba) into the solution in the interior of the membrane envelope. This is in addition to the Subdue added to the circulating solution at a rate of 1.2 ml/gal in all trials.

SOLUTION PREPARATION AND CHANGING:

The length of time the membrane has been reported to remain intact, i. e., before its integrity is compromised by microbial colonization, ranges from 20 to 30 days (13). With an intended ultimate stress level of -1.5 MPa, the water potential of the PEG solution was

stepped down 0.3 MPa at each of the solution changes which occurred each 5th day. The concentration of PEG 10,000 used to obtain these water potentials followed the curve determined by Castonguay (1; Table 1).

Table 1. PEG 10,000 concentrations used to obtain specified water potentials.

Water Potential (-MPa)	g PEG 10,000 / liter of solution
0.3	167
0.6	240
0.9	299
1.2	342
1.5	367

Sufficient solution was mixed at one time for both PEG and control treatments using stock solutions and distilled water. The solutions were agitated using a submersible pump (Little Giant) for a minimum of 30 minutes to ensure all components were dissolved before dispensing to the tubs or the addition of the PEG. The portion to be used for the PEG solution was transferred to a second container to which the PEG 10,000 was added and the mixture was stirred by hand using a wooden lath until the PEG was dissolved, when it was pumped to the appropriate tub. The transfer was completed using a marine (bilge) pump (Teel, Model 1P579F) with tubing of appropriate lengths attached to the intake and outflow ports. Use of the pump for this and other solution transfer steps eliminated the necessity of lifting large containers and the use of funnels, as well as speeded up the removal of solutions from the troughs.

With the solutions pre-mixed, the time from turning off the pumps until they were turned back on following the evacuation of the old solutions, the rinsing of the tubs and the refilling with fresh solution required 15 to 20 minutes for four troughs and two tubs. Because only sufficient tape was peeled back from the troughs to allow the insertion of a hose, the danger of adverse affects due to excessive evaporation from the surfaces of the membranes (or bare root systems) was considered to be negligible.

Although the volume and viscosity of the used solutions make disposal cumbersome and potentially messy, due to the relatively low concentrations of fungicide and the non-toxic nature of the PEG, special disposal methods are not required. If disposing of the solutions via the municipal sewer system copious amounts of water will be necessary to flush the drain of PEG.

TIME LINE OF EXPERIMENTS AND FINDINGS:

Fall 1993: ***Preliminary work***.

- ***Evaluated possibilities for containers, individual and multiple plant options.*** Settled on multiple plant options for speed of ease of changing and to avoid individual variation. Experimented with HDPE storage boxes as containers (Cole Parmer #M-06810-75 and -70). The three dimensionality of the boxes becomes problematic in maintaining low per plant volumes of the PEG solution. The use of weighted items to displace solution resulted in significant additional complications during solution changes.
- ***Ordered membranes, PEG, various other system components, and clonal plants for testing***. When clonal red and Freeman maple cultivars arrived they were discovered to have taken on fall coloration and formed terminal buds. They were placed in cold storage to fulfill their chilling requirements for breaking dormancy.
- ***Experimented with conditions required to grow healthy plants in hydroponic system***. Determined, using aquarium airstones, that continuous solution aeration, although it improved plant growth somewhat, was not necessary if solutions were changed within five days. Since this is the time recommended (1) for nutrient solution replacement to maintain acceptable pH and nutrient levels, no aeration mechanism other than having the solution cascade into a holding tub were designed into the set-up.

  Determined that Banrot (Grace-Sierra) supplied as a wettable powder to the hydroponics solutions did not provide adequate microbial control. Use of agitation may increase effectiveness, otherwise a soluble fungicide and/ or bactericide is necessary. No chemicals were found expressly for use in hydroponic systems. Determining rates to avoid phytotoxicity under these chronic exposure conditions and yet attain effective microbe control remains an elusive goal, significantly hindering the progress of this project. Many of the microbes that degrade the cellulose membranes are present on the plant roots before they are placed in the hydroponic system and thus the control chemicals need to be directed especially to the interiors of the membrane pouches. The composition of the biota collected from colonized membranes was analyzed by a university plant pathologist. Most of the organisms are not plant pathogens and much of the growth is bacterial, which is not controlled by fungicides. Some of the film which accumulates on the interior surface of the membranes may be due to root exudates which would not be noticed in a solid medium or in a free flowing hydroponic system.
- ***Experimented with options for sealing membrane envelopes***. The weighted dialysis tube closures sold by the distributors of the membranes (#29-0596-17 PGC Scientific, Gaithersburg, MD) provide inadequate length to close the clamps without folding the membrane longitudinally. The added and uneven thickness of the folded membranes caused a bowing of the clamps and leakage.

January - March 1994: ***Trial I, Prototype troughs and Boxelder seedlings.***

- ***Prototype trough construction and preliminary trial with*** **Acer negundo**. Built two prototype tanks from 1/4" plexiglas fused with methylchloride. Acquired access

to a controlled environment chamber which provides the advantage of reproducible temperature and light conditions. The temperature control system failed, however, and the plants were chilled to near freezing for 16 hours. Both tanks developed leaks, one serious enough to require it to be emptied of plants and solution. Without a control it was not possible to determine if the symptoms which developed on the seedlings were the result of chilling, drought stress (which inhibits phosphorous translocation (12), or from taking up PEG. The plants were subjected to increasing low-water stress, down to ¯0.9 MPa. The membranes did not exclude the PEG and the plants' leaves became necrotic, with symptoms progressing inward from the tips and margins. They also exhibited significant purplish coloration in a species not known for its anthocyanin production, but which is consistent with phosphorous deficiency.

Considerable phenotypic variation was observed in this half-sib seedling group. The possibility that the bag leaks developed earlier for some individuals than for others renders it difficult to evaluate the significance of this observation. The youngest leaves continued to be pliable and turgid, though they expanded only slightly during the course of the experiment (18 days). Plexiglas, especially of this thickness, was determined to be an inappropriate material from which to construct the troughs due to its ability to absorb water and its tendency to warp.

Summer 1994: ***Interim.***

- ***Design and construction of the Phase II troughs and other system components***. Contacted the Scientific Apparatus service on campus to assist with the design and construction. Placed order in late May and received shipment in August.
- ***Grew clonal maples as test plant materials***. Removed from cold storage and placed in greenhouses in soil medium. Experienced difficulties with root pathogens and thrip infestations. Since leaf expansion was an attribute to be measured, it was important that deformation of the leaves by other factors be minimized or eliminated. The soil pathogens were also of great concern as root health and rate of new root growth was another characteristic we wished to evaluate in the hydroponic system. Plants were drenched with Banrot fungicide and kept fairly dry between waterings. To force more uniform growth, plants were cut back to two nodes.

September - October 1994: ***Trial II: Initial trial using professionally constructed troughs and maple clones.***

- ***Objectives.*** The goals of this trial were to determine: 1) if membrane integrity could be maintained for a minimum of three weeks using the anti-microbial compounds and practices incorporated in the system at that time, 2) if healthy plants could be maintained in the hydroponic system, and 3) if the system was sufficiently functional to be used without modification for drought stress induction at a later date.
- ***Methods.*** Plants were placed in treatment on September 1st. Twelve replicates of each of the four clones were included in the study. The clones used were; *Acer rubrum* 'Autumn Flame' and 'Red Sunset' and *Acer x freemanii* 'Autumn Blaze' and 'Indian Summer'. On September 7th, I unexpectedly was required to move the entire system to another greenhouse. Significant drying of the membranes occurred before solution could be restored to the plants after the move. Also, shared space in the

original location precluded the use of supplemental lighting for photoperiod modification. The full duration of the trial was 78 days.

- ***Results and conclusions.***

1) ***Membrane longevity***. By day 15, in spite of the addition of Subdue to the circulating solution and to the solution inside the membranes, colonies of microbes had formed on them and holes were apparent (Figure 7). Therefore, during the final month there were no effective membranes encasing the root systems. I concluded that additional measures needed to be taken to control bacteria and better control fungal growth, and that controlling (lowering) solution temperatures might help reduce microbe growth to an acceptable level as well as reduce plant stress.

1) ***Plant health***. ***I: Incipient dormancy***. -The plants began to assume fall coloration (Figure 8) and form terminal buds. Whether this resulted from insufficient daylength or from the drought experienced in the move, or from the effects of high solution temperatures (as high as 38°C), or a combination of factors was not discernible. The plants forming terminal buds earliest showed few symptoms of wilting following the drying from the move, compared with the actively growing individuals. Dormancy or quiescence is an effective drought response in grasses and may also be a mechanism employed by woody plants. This, however, introduces an additional complicating factor for drought testing. Porometer readings taken to gauge drought stress effects on gas exchange behavior may be misleading if plants are in various stages of incipient dormancy. It will be important to determine the critical daylength or other factor(s) triggering dormancy in each (or the most sensitive) of the cultivars in order to avoid having plants in differing physiological statuses during drought tolerance screening.

- ***II: Possible phytotoxicity***. The plants also exhibited a crisping of the leaves beginning with the lowest pair. It is possible that the concentration of fungicide used in the experiment was harmful to the plants or that pathogens caused the damage. If phytotoxicity is the culprit, genotype specific differences in leaf expansion and rate of root development thus become confounded with differential sensitivities to the anti-microbial compounds. System modifications such that cultural practices rather than chemicals maintain acceptable microbe levels, in addition to solution cooling need to be investigated.

- ***System functions***. The changing of solutions needs to be speeded up to avoid drying of the membranes between solutions when the full complement of tanks is used. The use of a siphoning pump was determined to be necessary as a result of this trial.

Winter 1994 - 1995: ***Trial III, Multifactorial using refined system***.

- ***Objectives.*** The primary intent of this trial was to retest the design of the screening equipment as modified, i. e., incorporation of a solution cooling system and insulation, a siphoning pump for transferring solutions, and use of a anti-bacterial compound inside of membrane envelope. Success would be determined by whether intact membranes and healthy control plants existed at the conclusion of the test.

Additional objectives were to test the effects of three treatments.

I. Tomatoes vs. Green Ash. Tomatoes would be used as a bioassay to determine if the level of oxygen reaching the root systems of bagged and/or PEG treated plants was sufficient. Tomatoes are reputed to be sensitive to low soil or solution oxygen levels and to have immediate and distinctive symptom expression of root hypoxia. If symptoms developed in tomatoes, the green ash, which may be less symptomatic, could also be assumed to be experiencing root hypoxia which can mimic drought stress in its effects on plants (2).

II. Bagged vs. Unbagged. To test for ourselves the conflicting reports about toxic effects of PEG if placed in direct contact with plant root systems, half of the plants would be left "unbagged", i. e., without membranes encasing the root system. If the effects of PEG were insignificant, genotypic differences in drought response could be detected in spite of PEG effects, and problems of membrane longevity would cease to be an impediment to the utility of the drought screen.

III. Provenance differences in drought tolerance of green ash seedlings. Seeds had been collected from 13 populations of Green Ash (*Fraxinus pennsylvanica* Marsh.) from disparate locations in Great Plains states by collaborators at the USDA Forest Service, Center for Semiarid Agroforestry, in Lincoln, Nebraska the previous season. Results from parallel drought screening studies being run in Lincoln would be used to determine if one or the other screening process was more effective in detecting drought tolerance, or if either screen was more likely to detect a specific mechanism evolved in plants to tolerate drought.

- ***Methods.***
- ***Plant Materials.***

On November 14th and 15th, 22 to 25 green ash embryos from each of the 13 seed sources were excised from their pericarps and integuments, and germinated in damp paper towels for 3 days prior to planting. Germinated seedlings were planted in a mixture of 50% perlite and 50% vermiculite in 2 7/8" square and 5 1/2" deep pots (Anderson Dye & Manufacturing Co., Portland, OR). The plants were labeled, randomized, and placed under 16-hour lighting. The plants were irrigated once daily, alternately with water and Peters' Excel 21-5-20 at a rate of 200 ppm of nitrogen.

Tomatoes (*Lycopersicum esculentum* 'Rutgers'; Bentley Seed Co., Cambridge, NY) were sown on two different dates in order to have plants of a size appropriate to be used in the trough system.

In late January 1995, it was observed that in spite of the 16 hour photoperiod, a number of the seedlings were ceasing to produce leaves and were forming terminal buds. By February 14, over half of the Wyoming and South Dakota ash had formed terminal buds (although none of the Kansas seedlings had done so). Also, at about the sixth set of true leaves some transition is made and compound leaves begin to be produced.

By the time the solution cooling system was completed in mid-March, the number of green ash that were still actively growing was insufficient to fill four troughs with six plants each. In order to have enough for two troughs, all but three plants were used and provenances were bulked. The size of the ash root systems was larger than optimal for use in the troughs. Some of the results may be explained by the damage done to the roots during insertion between the walls of the troughs.

Twelve plants each of green ash and tomatoes were randomized with respect to trough position and treatments, bagged vs. unbagged and drought stressed vs. control. The plants were rinsed of rooting media and dipped in a solution containing Phyton 27. They were then placed either inside membrane envelopes or directly into the nutrient solution.

For 72 hours prior to the application of the initial drought stress, both troughs were filled with the control solution (nutrients and Subdue fungicide) from one tub to allow the plants to recover from the shock of transplanting. On the third day the troughs were connected to separate reservoirs and the solutions were changed, with the first level of PEG (Table 1) included in one of the solutions. Solution changes incorporating increases in PEG in the drought stress trough were made 5 and 10 days later. All plants in the greenhouse were exposed to Pyrethrum PT1100 applied as an aerosol on day 3. The experiment was concluded on the eighteenth day from set-up.

- Observations, complications, and conclusions.

System functionality. Pumps and tubing were able to handle the viscosity of the -0.9 MPa solution. The slowness of the refilling of the PEG tank relative to the control indicated that in spite of the flowmeter readings, the exchange rate may have differed for the two treatments. The use of a siphoning pump enabled one person to change solutions within an acceptable time period.

The solution temperature control system did not function satisfactorily. The solutions attained temperatures below (9°C) and above (25°C) the set point of 20°C during various periods during the experiment. The two tanks also were at significantly different temperatures more than once, such that chilling effects could not be ruled out as an explanation for some of the symptoms exhibited by the tomatoes in the control trough. An attempt to use one thermostat to regulate cooling of both troughs also resulted in disparate temperatures due, presumably, to differences in heat transfer properties between the control and the PEG solutions.

Membrane integrity. Microbial growth on the membranes was observed as early as the fourth day of the experiment, became considerable by the 6th (Figure 9), and resulted in bag disintegration by day 13 or earlier.

Health of control plants. Control plants were not maintained in a healthy condition throughout the course of the study. Various, inconsistent symptoms rendered them inappropriate for use in gauging treatment effects.

Plant responses.

A. Tomatoes. The tomatoes showed considerable transplantation shock and may also have been damaged by the insecticidal spray. They exhibited black necrotic spots, however, even before drought stress of the insecticide was applied (Figure 10). Foliar symptoms were not qualitatively different between PEG (Figure 11) and control (Figure 12) plants. Root development continued, however, and expansion of leaves in the control plants appeared to exceed that of those in the PEG treatment. Wilt symptoms did not occur (after the initial transplant recovery) until the -0.9 MPa solution was applied. Within the PEG treatment, even at the highest level of drought stress, some unbagged tomatoes remained turgid while others wilted. In spite of

considerable amounts of microbial growth in the root zone, some bagged tomato plants from the PEG treatment exhibited considerable root growth.

B. Green Ash. No clear treatment effects could be detected. The plant responses ranged from almost immediate wilt and desiccation (Figure 9), to necrotic spots and interveinal chlorosis (Figure 13; left foreground and background), to the maintenance of healthy green foliage (Figure 13; right background) for the duration of the study.

The desiccated plants were both from the PEG treatment, but appeared never to have recovered from transplantation shock. The chlorosis appeared in both drought stressed and control plants and may have been more pronounced in bagged vs. unbagged individuals. These symptoms may result from the uptake of the bactericide through damaged root systems or from a nutrient deficiency or toxicity induced by the copper compounds contained in the Phyton 27.

It is impossible to determine unequivocally why the one unbagged control plant survived so well. At the end of the experiment it was determined that the apical meristem was not active, indicating that, as with the maples in Trial II, quiescence may enable plants to survive adverse conditions. In this case, since no drought stress was applied, the adverse conditions would be root injury and exposure to various potentially phytotoxic compounds. The other two unbaggged ashes had developing leaves at the start of the study, which died during the course of the experiment. The root systems of both the bagged and unbagged control plants, including the presumably quiescent one, had large numbers of decomposing as well as new roots. The new roots were courser in texture and less branched than those that had formed in the perlite/ vermiculite rooting medium (Figure 14).

CONCLUSIONS:

1. The drought screening system, as designed and operated does not enable the detection of genotypic differences in drought tolerance.

2. We cannot detect treatment effects or assess the impact of PEG uptake on plant health without the capability to maintain membrane integrity.

3. Consistent solution temperature control within and among troughs is necessary to achieve #2.

4. It will be necessary to eliminate, as far as is possible, other sources of harmful effects on experimental plants (e. g., insecticidal sprays, damaged root systems, and anti-microbial chemicals) in order to gauge treatment effects. (See recommendations below.)

RECOMMENDED DESIGN CHANGES FOR FURTHER STUDY:

*** Include some porous medium that the roots have become established in inside the dialysis membrane.**

Benefits:

- This enables the use of a wider selection of antimicrobial compounds, including those available only as wettable powders.
- Root aeration can occur through the soil column, and thus is not dependent upon aeration of the solution or diffusion of oxygen through the solution.
- Roots will have additional shading, rendering light leakage less of a problem.
- Root morphology would more closely approximate that found in plants growing in soil, whose conditions we are attempting to approximate.
- Plants being put into experiments would be subject to considerably less root damage and breakage than in a system where their roots are rinsed of media immediately prior to placement in the screening system.
- If then covered with a double layer of membrane as explained below, a simple and effect means of providing a water-proof seal of the membrane would be achieved.
- Surviving plants could be replanted for further observations without additional transplantation shock or acclimation period required.

Implementation:

- Grow plants in root trainers with a diameter slightly less than that of the membrane used (2 cm) until well established. Drench soil volume with appropriate broad spectrum fungicide(s) during week preceding experiment initiation.
- Have troughs designed as an linear array with holes cut in a piece forming a lid at the top. The diameter of the holes should be just slightly larger than the root trainer at the point where it rests in the support stand.
- Cut membranes ca. 2.2 times the height of the root trainers. After soaking, twist the membrane in the middle and fold back on itself to form a double layered tube. Or, if using a membrane with a smaller width, closures designed for use with membrane by manufacturer could be used effectively on a single layer, folded up 1 to 2 cm.
- Use a shallow bladed saw (such as is used to remove plaster casts) to make 4 cuts through the root trainers (Figure 15): A) around perimeter near base, B) around perimeter a short distance below the rim upon which the container rests, and C/D) longitudinally down sides between cuts A and B on opposite sides of the container.
- Remove bottom. Holding root mass intact, place tube, media, and plant inside prepared membrane and carefully retract the pieces cut from the sides. Place unit in rack, using friction to hold membrane between remaining rim of root trainer and edge of rack hole. Alternatively, use a nylon net liner sewn to fit a root trainer or other container. The liner can be used to remove the root mass relatively intact from the container and be placed in the membrane.

*** Construct extra troughs that fit the lids described above.**

Benefits:

- The lids, with plants placed in them, can be moved from tank to one with new solution in one motion. This reduces time required to change solutions and the possibility of drying between solutions.
- Troughs can be thoroughly rinsed between solutions, which would assist in controlling microbe populations.

*** Replace Subdue 2E with a Benlate/ Captan mixture.**

Benefits:

- In spite of the controversy surrounding Benlate toxicity, the use of this combination of fungicides appears to provide greater protection of membrane integrity than that provided by Subdue 2E, alone or in combination with Phyton 27. (20-25 days or more (13,14), versus less than 10 days)

Implementation:

- The circulation system already in use to maintain solution temperature control and solution aeration levels can be utilized with an increased exchange rate to maintain the wettable powders in suspension and/or circulate whatever portion of the active ingredient is soluble to the membrane surface.
- The sizes of the holes in the tube dispensing solution at the bottom of the troughs may need to be increased to avoid clogging.
- Screens may need to be placed in the line between the solution reservoir tubs and the pumps to protect the pumps from damage by clay particles.

*** Time experiment to occur when seedlings are 4 to 8 weeks old from germination.**

Benefits:

- Although leaf area may be less than is desirable for taking gas exchange data, plants at this age will most likely be actively growing, i. e., probably not be entering quiescence. Extended day length is not sufficient to maintain active growth in some plant, i. e., they have determinate growth patterns.

*** Investigate the use of non-organic semipermeable membranes**.

Benefits:

- High costs may be offset by greater flexibility in experimental design and more accurate findings.

LITERATURE CITED:

1. Castonguay, Y. 1989. Stomatal and nonstomatal limitations to photosynthesis in *Phaseolus vulgaris* L. and *P. acutifolius* Gray in response to water stress. Ph. D. Thesis, University of Minnesota, Saint Paul, MN.

2. Gingrich, J. R. and M. B. Russell. 1956. Effect of soil moisture tension and oxygen concentration on the growth of corn roots. Agronomy Journal 48: 517-520.

3. Graham-Bryce, I. J. 1967. Method of supplying water to soil at osmotically controlled potentials. Chem. Ind. (London) 9: 353-354.

4. Graves, W. R. and L. C. Wilkins. 1991. Growth of honey locust seedlings during high root-zone temperature and osmotic stress. HortScience 26: 1312-1315.

5. Janes, B. E. 1968. Effects of extended periods of osmotic stress on water relationships of pepper. Physiologia Plantarum 21: 334-345.

6. Jarvis, P. G. and M. S. Jarvis. 1965. The water relations of tree seedlings. V. Growth and root respiration in relation to osmotic potential of the root medium. p.167 In: B. Slavik (ed.)Water Stress in Plants. The Hague.

7. Lawlor, D. W. 1970. Absorption of polyethylene glycols by plants and their effects on plant growth. New Phytologist. 69: 501-513.

8. Lagerwerff, G. V., G. Ogata, and H. E. Eagle. 1961. Control of osmotic pressure of culture solutions with polyethylene glycol. Science 133: 1486-1487.

9. Lesham, B. 1966. Toxic effect of carbowaxes (Polyethylene glycol) on *Pinus halipensis* seedlings. Plant Soil 24: 322-324.

10. Merck Index.

11. Mexal, J. , J. T. Fisher, J. Osteryoung, and C. P. P. Reid. 1975. Oxygen availability in polyethylene glycol solutions and its implications in plant-water relations. Plant Physiology 55: 20-24.

12. Resnik, M. E. 1970. Effects of mannitol and polyethylene glycol on phosphorous uptake in maize. Annals of Botany 34: 497-505.

13. Sikurajapathy, M., J. J. Cappy, and H. D. Gross. 1983. A method for inducing controlled moisture stress on seedlings. Agronomy Journal 75: 840-843.

14. Tingey, D. T. and C. Stockwell. 1977. Semipermeable membrane system for subjecting plants to water stress. Plant Physiology 60: 58-60.

15. Zur, B. 1966. Osmotic control of the matric soil-water potential: I. Soil-water system. Soil Science 102: 394-398.

16. Zur, B. 1967. Osmotic control of the matric soil-water potential: II. Soil-plant system. Soil Science 103: 30-38.

Figure 1. Hydroponic system set up for Trial II. Solution is circulated through one reservoir tub.

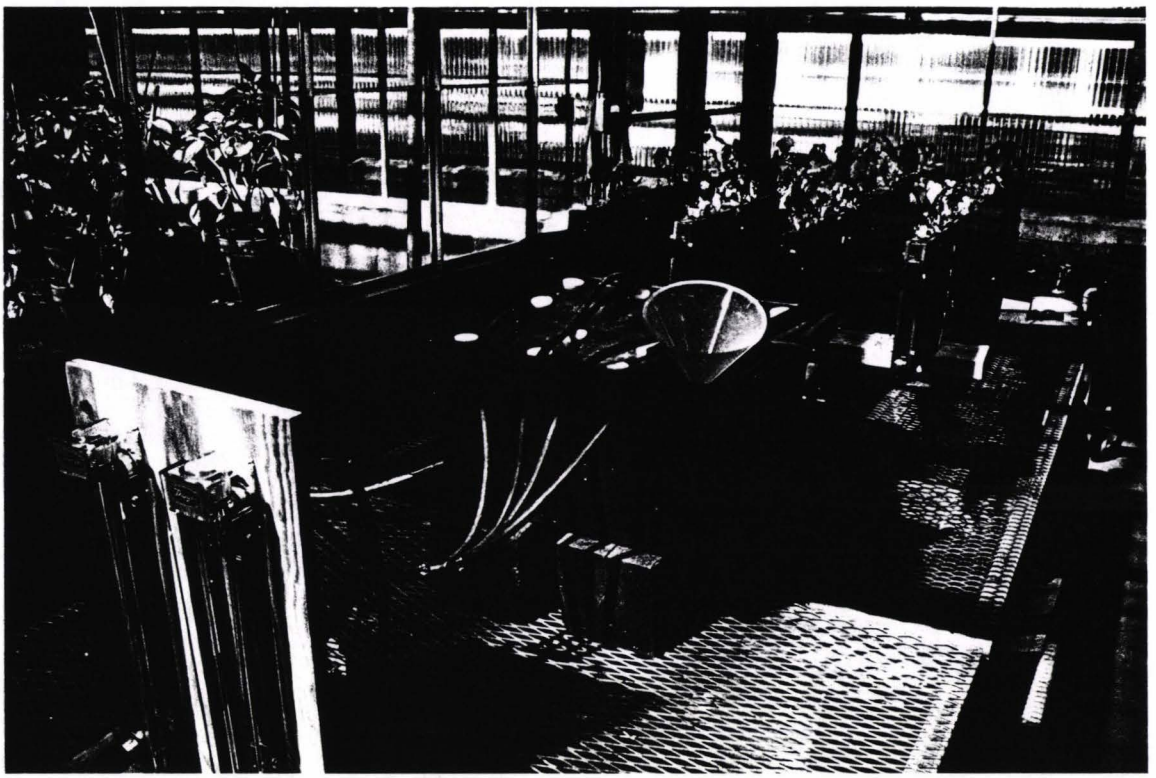

Figure 2. Modified system with solution temperature control and insulation installed.

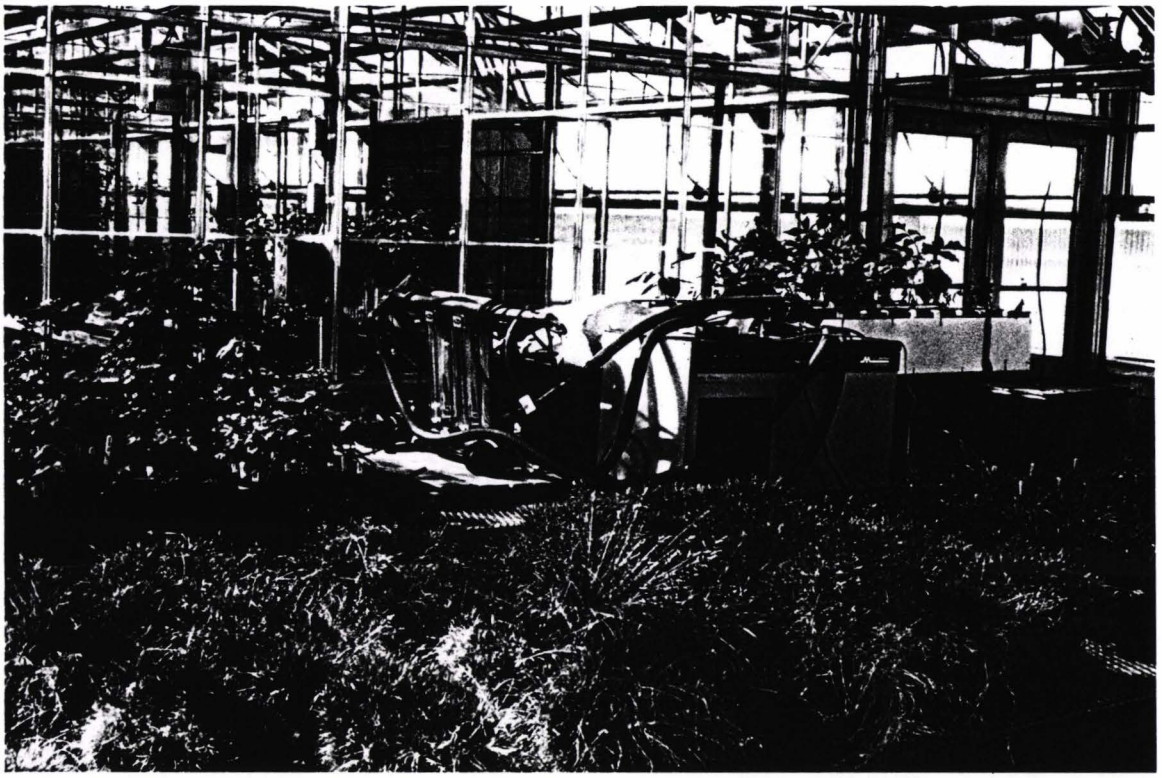

Figure 3. Heat exchangers fabricated to cool solutions.

Figure 4. Trough end showing insulation and support system. Spacers are also visible along top edge of trough.

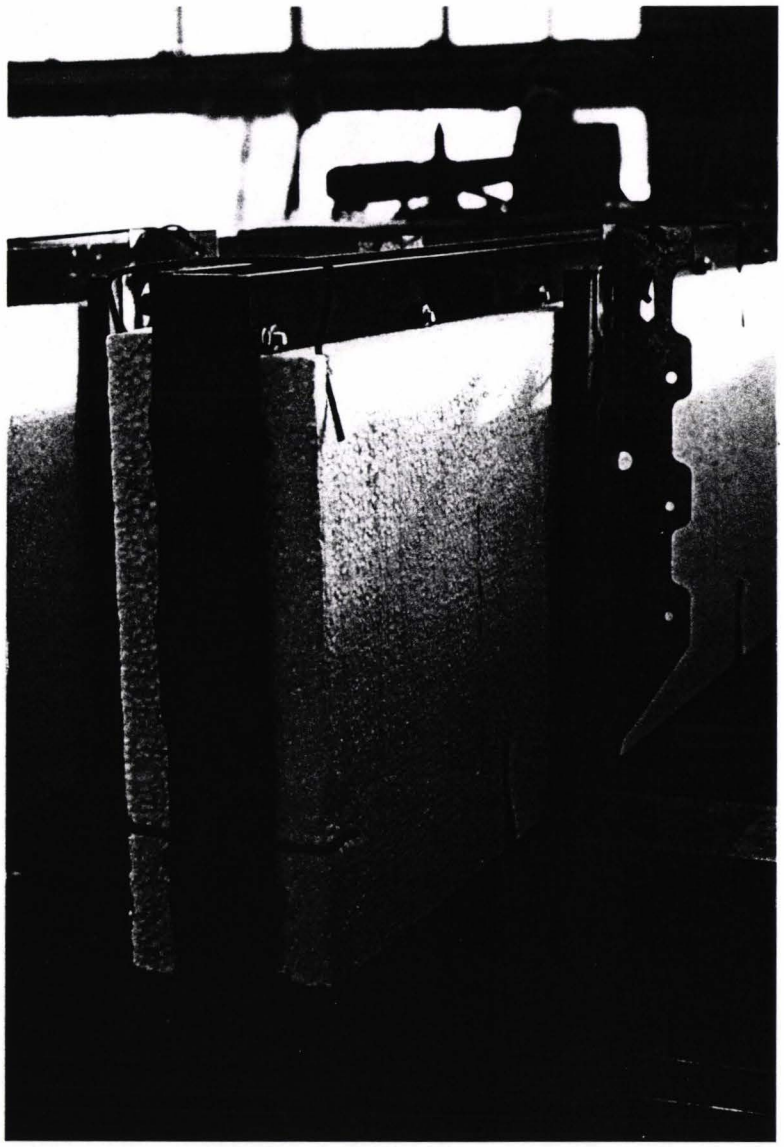

Figure 5. Sliced rod closure for membrane envelope, demonstrated here using plastic bag.

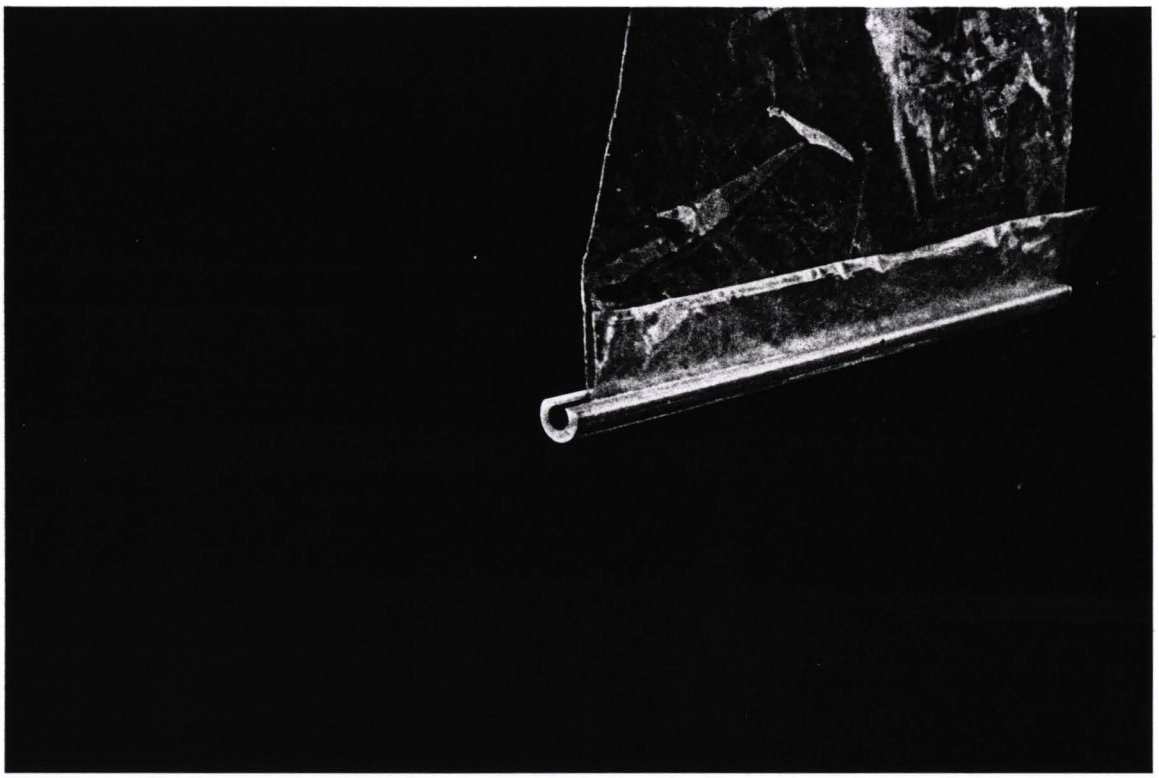

Figure 6. Stand holding complete plant-membrane assemblage.

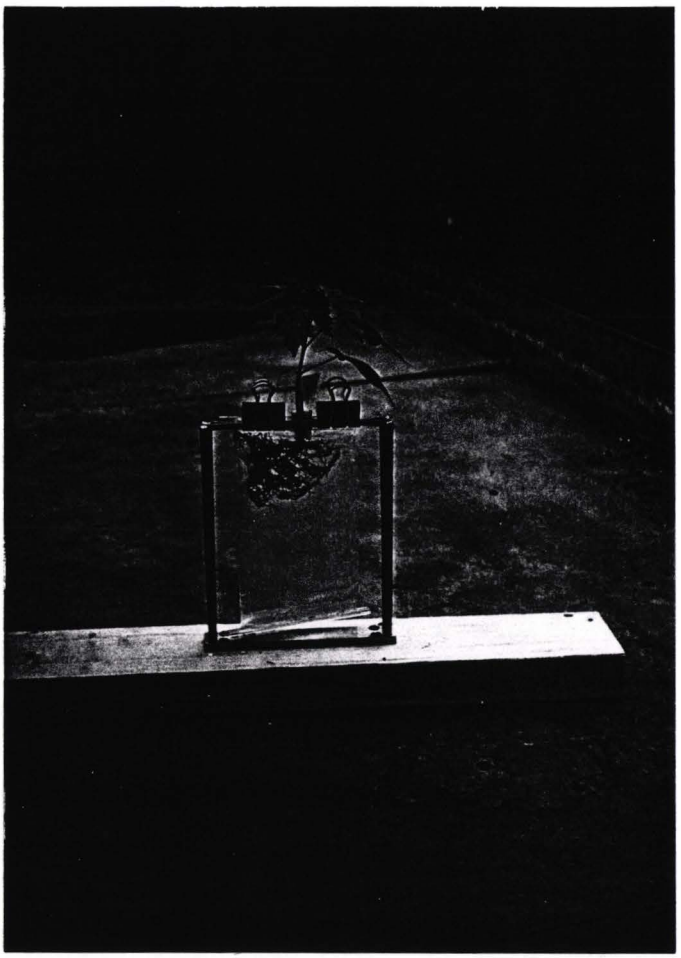

Figure 7. Colonies of microbes growing on cellulose membranes.

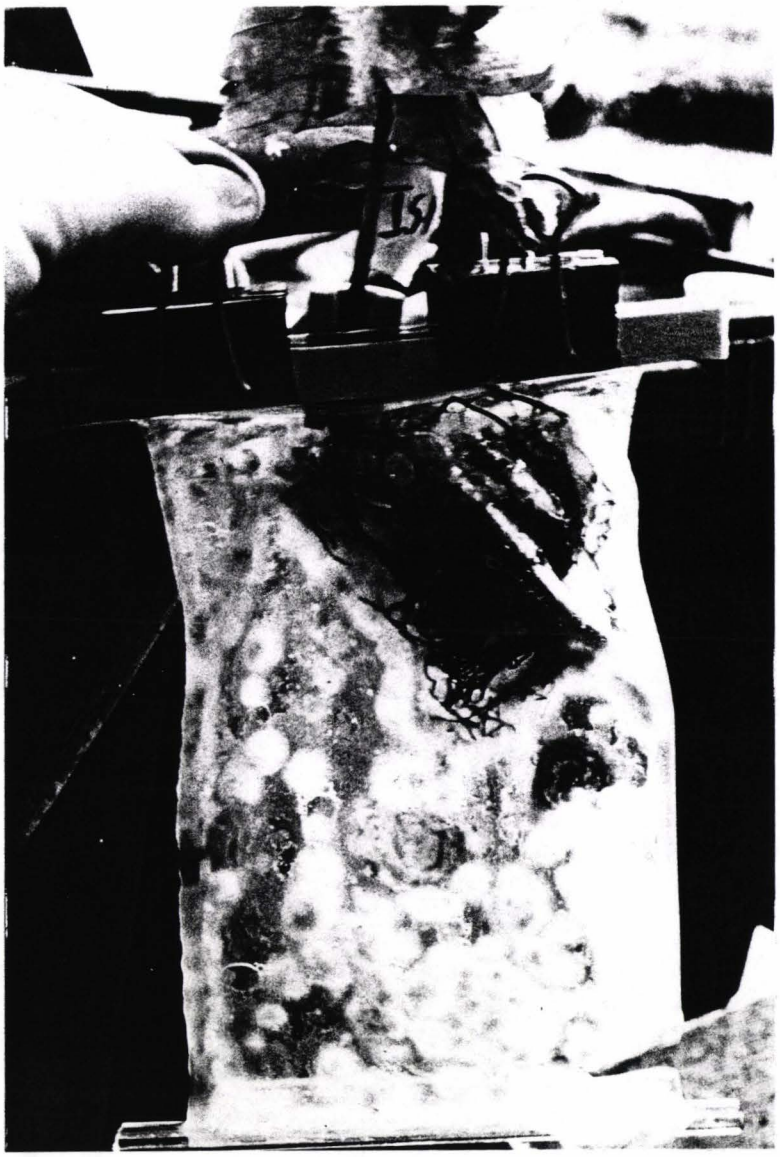

Figure 8. SJW and maples in Trail II showing fall coloration.

Figure 9. Bagged and unbagged ash seedlings on day 6 of experiment showing microbial growth on membrane and wilted, desiccated leaves.

Figure 10. Control tomato on day 2 of experiment showing black necrotic spots on foliage.

Figure 11. Tomato growing in PEG solution on day 13 of study.

Figure 12. Tomato growing in control solution on day 13 of study.

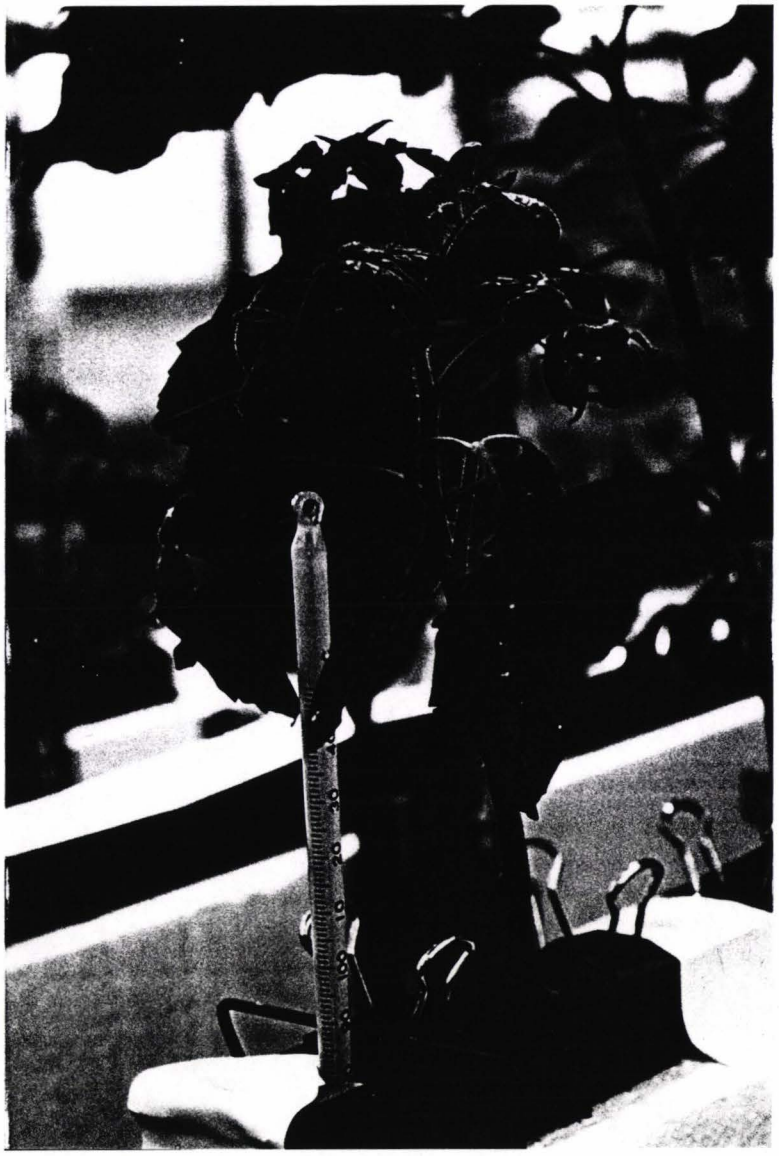

Figure 13. Plants on final day of experiment (day 18), with PEG trough in foreground.

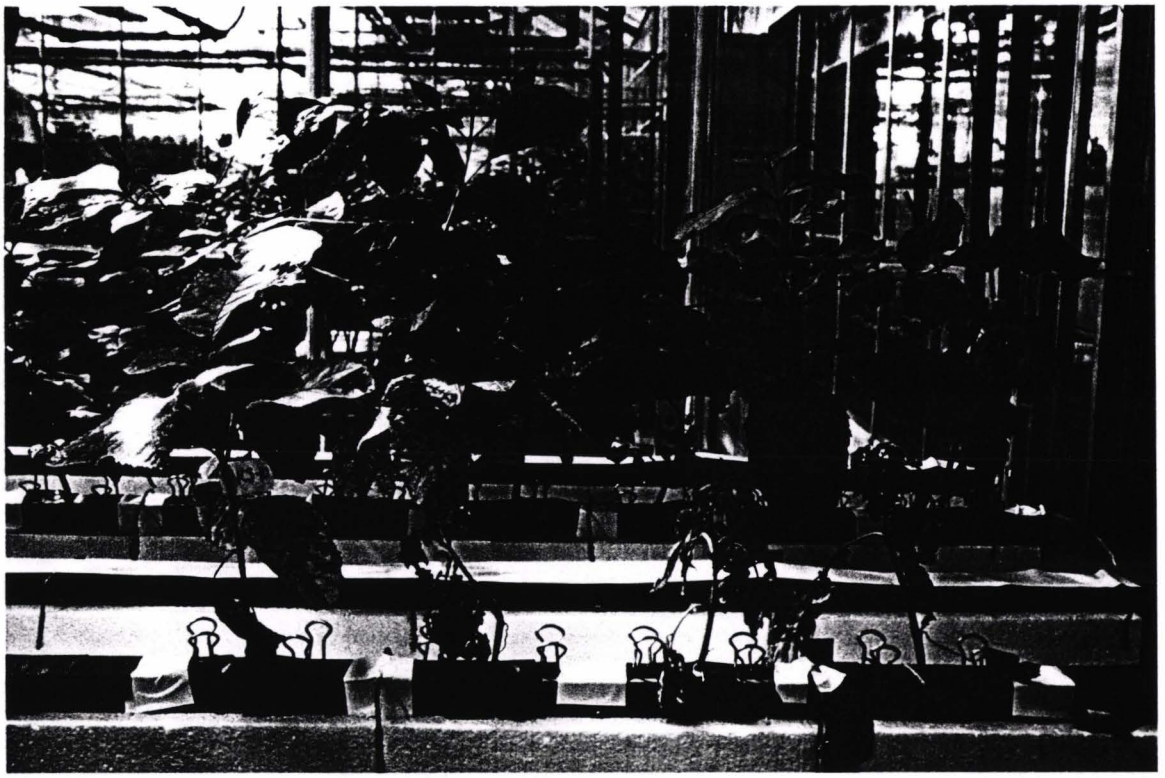

Figure 14. New root development and old root deterioration from healthiest unbagged green ash seedling in control treatment.